THE GENOMES OF THREE CORONAVIRUSES

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1. Introduction

A number of studies on the genomic RNAs of coronaviruses have been reported. The genome of avian infectious bronchitis virus (IBV) is a single single-stranded molecule of high mol. wt [1–4], which is prone to degradation into smaller molecules [1,5]. There is some disagreement concerning its mol. wt with estimates varying from 9.0×10^6 [1] to 5.6×10^6 [4]. Similar studies on the human coronavirus (HCV) genome have also shown it to be a large molecule of mol. wt varying from 6.1×10^6 for strain OC43 [6] to 5.8×10^6 for strain 229E [7]. Again, as with IBV, the HCV genome is prone to degradation [6,7]. The genomes of two porcine coronaviruses, transmissible gastroenteritis virus (TGEV) and haemagglutinating encephalomyelitis virus (HEV), have also been shown to be large single single-stranded RNA molecules of about 60 S that dissociate into 35 S and 4 S material on heating above 60°C [8]. Coronavirus genomes possess certain mRNA characteristics. Polyadenylic acid [poly(A)] sequences are found in the genomes of IBV [2,4,9] and HCV [7,10] at or near their 3′-termini [7]. Furthermore, the genome of IBV has been shown to be infectious [4,9] and no detectable virion transcriptase has been identified associated with IBV [4] or HCV [10] particles.

In this paper, studies on the characteristics of the genomes of HCV strain 229E (HCV 229E) and IBV strain Beaudette (IBV Beau) reported in [3,7] are extended, and the structure of the genome of another coronavirus, mouse hepatitis virus strain 3 (MHV 3) is analysed for the first time. A comparison of the results shows a much greater similarity between coronavirus genomes than has been previously observed.

2. Materials and methods

IBV Beau was grown in confluent primary chick kidney cell cultures and labelled with [^{3}H]uridine or [^{3}H]adenosine as in [3]. HCV 229E was grown in confluent monolayer cultures of continuous MRC cells and labelled with [^{3}H]uridine or [^{3}H]adenosine [7]. Finally, MHV 3 was grown in confluent secondary mouse embryonic fibroblasts. The cell monolayers were infected at an input multiplicity of 0.1 infectious particles per cell, with an adsorption period of 1.5 h at 37°C. At 6 h after infection, 20 μCi/ml of [^{3}H]-uridine or [^{3}H]adenosine (Radiochemical Centre, Amersham) were added to the medium. At 32 h after infection the cells were harvested and subjected to 3 freeze–thaw cycles. The virus preparations were purified on sucrose gradients and labelled virus particles with typical coronavirus morphology were obtained in peak fractions of density 1.18 g/ml coincident with peaks of infectivity [3,7].

RNA was extracted from coronavirus particles with proteinase K [7], purified on cellulose CF 11 columns and analysed on 2.2% polyacrylamide gels supported by 0.5% agarose [7]. Oligo(dT)-cellulose chromatography, digestion with pancreatic ribonuclease A and T_1 ribonuclease, and digestion with polynucleotide phosphorylase have been described in [7].

3. Results

Figure 1 shows typical profiles of purified [^{3}H]-uridine-labelled genomic RNA from IBV Beau (fig.1A), HCV 229E (fig.1B) and MHV 3 (fig.1C). In all cases a single peak of activity was obtained, corresponding to mol. wt ~5.8 X 10^6 by reference to cellular rRNAs, mol. wt 1.64 X 10^6 and 0.67 X 10^6 [11], and their 45 S precursor, mol. wt 4.1 X 10^6 [12]. As these RNA molecules are so large and their mol. wt were calculated by extrapolation of marker RNAs beyond that of the largest marker, their mol. wt may be over- or underestimated by an unknown amount. However, it is clear from these results that the sizes of these coronavirus genomes are almost identical.

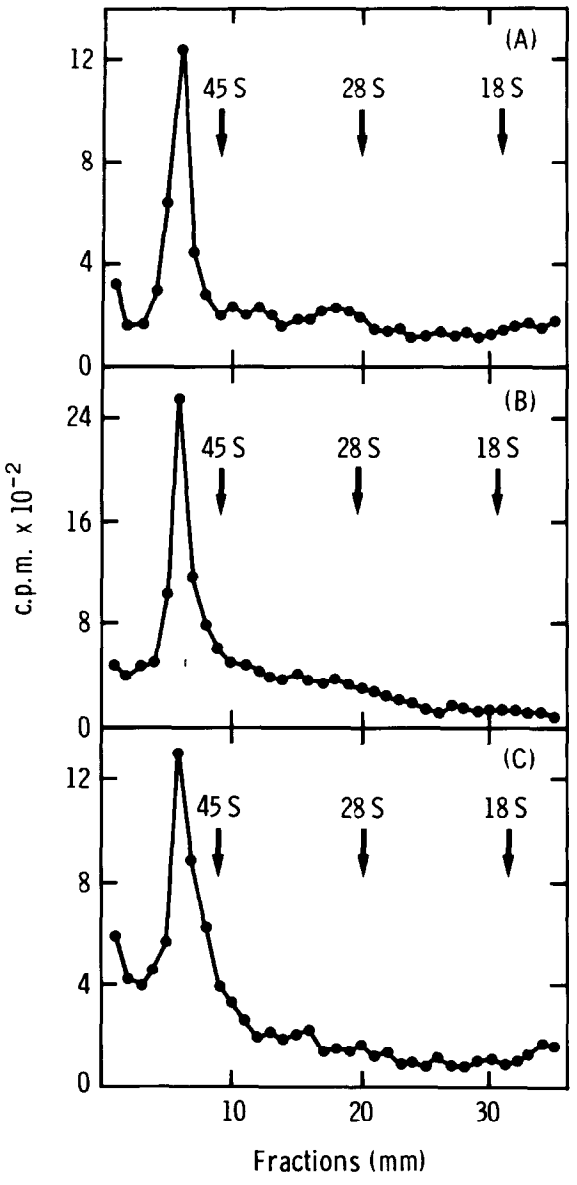

Fig.1. Electrophoresis on 2.2% polyacrylamide gels of coronavirus RNAs labelled with [^{3}H]uridine. (A) HCV 229E RNA. (B) IBV Beau RNA. (C) MHV 3 RNA. The arrows indicate the positions of unlabelled rRNA species which were co-electrophoresed on the same gels.

When these RNAs were denatured by heating with 10% formaldehyde for 20 min at 67°C [13], no difference in their migration on 2.2% polyacrylamide gels was observed. This shows that the coronavirus genomes are not composed of multiple subunits and contain little, if any, double strandedness. Some reports suggest that heating or denaturation can produce an alteration in the size or migration of the RNAs [5,6,8]. This may be due to contamination of the RNAs by an activated retrovirus or the presence of an internal ribonuclease [4] or the nicking of the large RNAs during isolation.

The presence of poly(A) tracts in the coronavirus genomes was investigated by binding purified RNA species to oligo(dT)-cellulose columns [7]. As shown in table 1, 30% of MHV 3 genome RNA, 29% of HCV 229E RNA and 27% of IBV Beau RNA bound to the columns. Under the same conditions of chromatography, poly(A) bound 100% and essentially no poly(U) or rRNA bound to the columns. These results indicate that ~25–30% of coronavirus genomic

Table 1
Binding of labelled polyribonucleotides to oligo(dT)-cellulose columns

Polyribonucleotide	% binding
Poly(A)	100
MHV 3 RNA	30[a]
HCV 229E RNA	29[a]
IBV Beau RNA	27[a]
rRNA	2
Poly(U)	0

[a] Average of 5 determinations ⩾ 12 000 cpm of [^{3}H]uridine- or [^{3}H]adenosine-labelled coronavirus RNA was used, of which ⩾ 3000 cpm bound to the oligo(dT)-cellulose columns

RNAs contain covalently attached poly(A) sequences that are able to bind to oligo(dT)-cellulose columns. Substantially more of the genomic RNA may be polyadenylated, but may not be able to bind to oligo(dT)-cellulose columns. This may be because the genomic RNAs are very large and some of them take up conformations during chromatography that prevent them from binding to the oligo(dT)-cellulose, or because the poly(A) content of the molecules is very heterogeneous and many of the molecules have poly(A) tracts that are too short to readily bind to the columns. There was no evidence suggesting that the RNA species were degraded during chromatography as single peaks of activity were obtained on polyacrylamide gels of chromatographed RNAs.

Labelled coronavirus RNA species that had bound to oligo(dT)-cellulose columns were digested with a mixture of pancreatic ribonuclease A and T_1 ribonuclease in order to obtain the poly(A) portions of the genomes and to size them. Table 2 shows the results of digestion of [^{3}H]uridine- and [^{3}H]adenosine-labelled coronavirus RNAs. About 1.6% of the [^{3}H]-adenosine-labelled RNAs were resistant to nuclease digestion. Assuming that ~1 in 4 nucleotides of the coronavirus genome is adenosine, then about 0.4% of the genome consists of poly(A). Essentially, there was no [^{3}H]uridine-labelled RNA ribonuclease resistant radioactivity, showing that the nuclease treatment had eliminated all the RNA sequences except the poly(A) tracts. The coronavirus genomes have been shown to have mol. wt $\sim 5.8 \times 10^6$ which corresponds to ~18 000 nucleotides. Assuming that 0.4% of the coronavirus genome is poly(A), then the poly(A) tract is about 70 nucleotides in length. [^{3}H]Adenosine-labelled poly(A) sequences were run on 5% polyacrylamide gels with tRNA as marker in order to obtain a direct estimate of their size [10]. However, no clear profile was obtained as the amount of label in the poly(A) was too low for such an analysis.

Controlled digestion with the enzyme polynucleotide phosphorylase was used to digest coronavirus genomic RNAs from the 3′-termini, in order to determine whether the poly(A) segment was located at the 3′-end of the genome. Table 3 shows that controlled digestion of the three species of coronavirus RNAs, HCV 229E, IBV Beau and MHV 3, with polynucleotide phosphorylase at 37°C for 10 min, produced a much more rapid loss of poly(A) than RNA. These results are interpreted to mean that the three coronavirus RNAs studied have poly(A) segments at or near the 3′-termini of the molecules.

Table 2
Binding of labelled coronavirus RNAs to oligo(dT)-cellulose columns after digestion with nucleases[a]

RNA[b]	Isotope	% binding[c]
MHV 3	[^{3}H]Uridine	0
HCV 229E		0.1
IBV Beau		0.1
MHV 3	[^{3}H]Adenosine	1.6
HCV 229E		1.6
IBV Beau		1.5

[a] Digestion was at 37°C for 30 min in a solution containing 200 mM NaCl, 20 mM Tris–HCl (pH 7.5), 10 μg/ml pancreatic ribonuclease A and 30 units/ml T_1 ribonuclease

[b] Only RNA species binding to oligo(dT)-cellulose were used. The initial cpm before nuclease digestion were ⩾ 20 000 in all cases

[c] Each experiment was an average of 3 determinations

Table 3
Digestion of coronavirus RNAs with polynucleotide phosphorylase[a]

RNA species[b]	% total RNA remaining[c]	% poly(A) remaining[d]
HCV 229E	75	30
IBV Beau	81	35
MHV 3	84	21

[a] Samples were incubated at 37°C for 10 min in a solution containing 500 μg/ml polynucleotide phosphorylase in 0.1 M Tris–HCl (pH 8.5), 5 mM $MgCl_2$, 10 mM sodium phosphate. Incubation without enzyme or sodium phosphate produced no digestion

[b] Only RNA species binding to oligo(dT)-cellulose were used. The initial cpm before digestion were ⩾ 10 000 in all cases

[c] Each experiment was an average of 2 determinations. The amount of RNA remaining after digestion was determined by trichloroacetic acid precipitation

[d] Each experiment was an average of 2 determinations. The amount of poly(A) remaining after digestion was determined by the ability of the RNA species to bind to oligo(dT)-cellulose columns

4. Discussion

The results presented in this paper show that the genomes of HCV 229E, IBV Beau and MHV 3 are all single single-stranded molecules of mol. wt ~5.8 × 10^6. Furthermore, at least 25–30% of these RNAs contain covalently-bound poly(A) tracts of about 70 nucleotides in length located at or near the 3′-termini of the molecules. This and previous data [3,4,7,9,10], together with data showing that these genomes are infectious [4,9] and that there is no virion transcriptase [4,10], strongly suggest that the coronavirus genome is of positive polarity. These results are important for a number of reasons. Firstly, they confirm and extend reports on the genomes of IBV and HCV concerning their structure and polarity. Secondly, they extend our knowledge to include the structure of the genome of another coronavirus, MHV. Finally, the results show a much greater similarity between coronavirus genomes than has been previously reported. Previous discrepancies may be due in part to the varying methods used for the analysis of the genomic RNAs in different laboratories.

During the preparation of this paper, a report was published [14], showing that two other MHV strains, A59 and JHM, have polyadenylated sequences of mol. wt 5.4 × 10^6. However, the location and size of these poly(A) sequences was not determined.

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References

[1] Watkins, H., Reeve, P. and Alexander, D. J. (1975) Arch. Virol. 47, 279–288.
[2] Lomniczi, B. and Kennedy, I. (1977) J. Virol. 24, 99–107.
[3] Macnaughton, M. R. and Madge, H. M. (1977) FEBS Lett. 77, 311–313.
[4] Schochetman, G., Stevens, R. H. and Simpson, R. W. (1977) Virology 77, 772–782.
[5] Tannock, G. A. (1973) Arch. Ges. Virusforsch. 43, 259–271.
[6] Tannock, G. A. and Hierholzer, J. C. (1977) Virology 78, 500–510.
[7] Macnaughton, M. R. and Madge, H. M. (1978) J. Gen. Virol. 39, 497–504.
[8] Garwes, D. J., Pocock, D. H. and Wijaszka, T. M. (1975) Nature 257, 508–510.
[9] Lomniczi, B. (1977) J. Gen. Virol. 36, 531–533.
[10] Tannock, G. A. and Hierholzer, J. C. (1978) J. Gen. Virol. 39, 29–39.
[11] Peterman, M. L. and Pavlovec, A. (1966) Biochim. Biophys. Acta 114, 264–276.
[12] Weinberg, R. A. and Penman, S. (1970) J. Mol. Biol. 47, 169–178.
[13] Macnaughton, M. R., Freeman, K. B. and Bishop, J. O. (1974) Cell 1, 117–125.
[14] Lai, M. M. C. and Stohlman, S. A. (1978) J. Virol. 26, 236–242.